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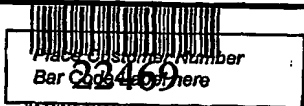
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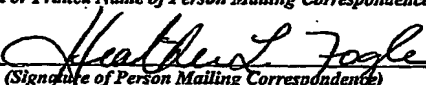
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Blockade of Rat $\alpha 3 \beta 4$ Nicotinic Receptor Function by Methadone, Its Metabolites, and Structural Analogs

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ABSTRACT

The opioid agonist properties of (\pm)-methadone are ascribed almost entirely to the (–)-methadone enantiomer. To extend our knowledge of the pharmacological actions of methadone at ligand-gated ion channels, we investigated the effects of the two enantiomers of methadone and its metabolites *R*-(+)-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP) and *R*-(+)-2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline hydrochloride (EMDP), as well as structural analogs of methadone, including (–)- α -acetylmethadol hydrochloride (LAAM) and (+)- α -propoxyphene, on rat $\alpha 3 \beta 4$ neuronal nicotinic acetylcholine receptors (nAChRs) stably expressed in a human embryonic kidney 293 cell line, designated KX $\alpha 3 \beta 4$ R2. (\pm)-Methadone inhibited nicotine-stimulated $^{86}\text{Rb}^+$ efflux from the cells in a concentration-dependent manner with an IC_{50} value of $1.9 \pm 0.2 \mu\text{M}$, indicating that it is a potent nAChR antagonist. The (–)- and (+)-enantiomers of methadone have similar inhibitory po-

tencies on nicotine-stimulated $^{86}\text{Rb}^+$ efflux, with IC_{50} values of approximately $2 \mu\text{M}$. EDDP, the major metabolite of methadone, is even more potent, with an IC_{50} value of approximately $0.5 \mu\text{M}$, making it one of the most potent nicotinic receptor blockers reported. In the presence of (\pm)-methadone, EDDP, or LAAM, the maximum nicotine-stimulated $^{86}\text{Rb}^+$ efflux was markedly decreased, but the EC_{50} value for nicotine stimulation was altered only slightly, if at all, indicating that these compounds block $\alpha 3 \beta 4$ nicotinic receptor function by a noncompetitive mechanism. Consistent with a noncompetitive mechanism, (\pm)-methadone, its metabolites, and structural analogs have very low affinity for nicotinic receptor agonist binding sites in membrane homogenates from KX $\alpha 3 \beta 4$ R2 cells. We conclude that both enantiomers of methadone and its metabolites as well as LAAM and (+)- α -propoxyphene are potent noncompetitive antagonists of $\alpha 3 \beta 4$ nAChRs.

Nicotinic acetylcholine receptors are distributed throughout the central and peripheral nervous systems where they mediate the actions of endogenous acetylcholine, as well as nicotine and other nicotinic agonists. They are often associated with cell bodies and axons of major neurotransmitter systems, and nicotinic agonists are thought to act through these receptors to promote the release of a number of neurotransmitters such as dopamine, norepinephrine, γ -aminobutyric acid, acetylcholine, and glutamate (for review, see Wonnacott, 1997), as well as certain pituitary hormones (Andersson et al., 1983; Sharp et al., 1987; Flores et al., 1989; Hulihan-Giblin et al., 1990). The release of this wide array of neurotransmitters and hormones probably contributes to the diverse, and sometimes opposite, effects of nicotine. For ex-

ample, the release of norepinephrine is usually associated with arousal, while the stimulation of γ -aminobutyric acid systems is associated with sedation.

Nicotine was first examined for its potential as an analgesic drug almost 70 years ago (Davis et al., 1932), but its dose-response relationship for analgesia yielded a poor therapeutic index, which did not favor its development. More recently, following the discovery of the analgesic properties of epibatidine, a potent nicotinic agonist isolated from the skin of an Ecuadorian frog by Daly and colleagues (Spande et al., 1992), there has been renewed interest in the analgesic potential of drugs that act at nicotinic receptors (Bannon et al., 1998; Flores and Hargreaves, 1998; Flores, 2000).

It is likely that more than one neurotransmitter system plays an important role in analgesia. For example, methadone, a synthetic μ -opioid agonist, has analgesic properties similar to morphine's (Kristensen et al., 1995), and it is also useful in the treatment of opiate addiction. Most of the morphine-like analgesic properties of (\pm)-methadone are as-

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; EB, (\pm)-epibatidine; LAAM, (–)- α -acetylmethadol hydrochloride; (+)-EDDP, *R*-(+)-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate; (–)-EDDP, *S*-(–)-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate; (+)-EMDP, *R*-(+)-2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline hydrochloride; (–)-EMDP, *S*-(–)-2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline hydrochloride; nAChR, nicotinic acetylcholine receptor.

cribed to the (-)-enantiomer, since the (+)-enantiomer has much weaker opiate properties (Scott et al., 1948; Smits and Myers, 1974; Horng et al., 1976). However, (+)-methadone does show analgesic potency in some experimental models (Shimoyama et al., 1997; Davis and Inturrisi, 1999), and it also appears to attenuate development of morphine tolerance (Davis and Inturrisi, 1999).

In addition to its agonist action at opiate receptors, methadone competes for [³H]MK801 binding sites within the NMDA receptor channel and blocks NMDA receptor-mediated responses (Ebert et al., 1995); furthermore, the two enantiomers of methadone are nearly equipotent at [³H]MK801 binding sites (Gorman et al., 1997). Several drugs such as MK801, phencyclidine, dextromethorphan, and dextrorphan that block NMDA receptors also block neuronal nicotinic receptors (Ramos et al., 1990; Amador and Dani, 1991; Hernandez et al., 2000). Both nicotinic receptors and NMDA receptors have been implicated in pain pathways and possible mechanisms underlying the perception of pain. Therefore, we examined the effects of methadone, its metabolites, and structural analogs (Fig. 1) on neuronal nicotinic receptors. To do this, we measured the actions of these compounds at $\alpha 3 \beta 4$ neuronal nicotinic receptors stably expressed in human embryonic kidney 293 cells. We found that these drugs are potent nicotinic receptor blockers; in fact, one of the methadone metabolites is among the most potent nicotinic receptor blockers that have been reported.

Experimental Procedures

Materials and Drugs. Tissue culture medium, antibiotics, and serum were obtained from Invitrogen (Carlsbad, CA). [³H](±)-epibatidine ([³H]EB) and [⁸⁶Rb]rubidium chloride (⁸⁶Rb⁺) were supplied by PerkinElmer Life Science Products (Boston, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. (±)-Methadone hydrochloride (methadone), S-(+)-methadone hydrochloride [(+)-methadone], and R-(-)-methadone hydrochloride [(-)-methadone] were obtained from Sigma/RBI (Natick, MA). The following compounds were generously provided by Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse: (-)- α -acetylmethadol hydrochloride (LAAM, a methadone analog); R-(+)-2-ethyl-1,6-dimethyl-3,3-diphenylpyrrolinium perchlorate [(+)-EDDP, a methadone metabolite]; S-(-)-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate [(-)-EDDP, a methadone metabolite]; R-(+)-2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline hydrochloride [(+)-EMDP, a methadone metabolite]; S-(-)-2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline hydrochloride [(-)-EMDP, a methadone metabolite]; (+)- α -propoxyphene hydrochloride (a methadone analog); and (+)- α -N-norpropoxyphene maleate (a propoxyphene metabolite). The structures of methadone, its metabolites, and structural analogs used here are shown in Fig. 1, along with mecamylamine, a well known nicotinic channel blocker.

Cell Culture. The cell line KKA3 β 4R2 was established previously by stably cotransfecting human embryonic kidney 293 cells with the rat $\alpha 3$ and $\beta 4$ nAChR subunits genes (Xiao et al., 1998). Cells were maintained in minimum essential medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, 100 mg/ml streptomycin, and 0.7 mg/ml of geneticin (G418) at 37°C with 5% CO₂ in a humidified incubator.

⁸⁶Rb⁺ Efflux Assay. Function of nAChRs expressed in the transfected cells was measured using a ⁸⁶Rb⁺ efflux assay as described previously (Xiao et al., 1998). In brief, cells in the selection growth medium were plated into 24-well plates coated with poly(D-lysine). The plated cells were grown at 37°C for 18 to 24 h to reach 70 to 95% confluence. The cells were then incubated in growth medium (0.5 ml/well) containing ⁸⁶Rb⁺ (2 μ Ci/ml) for 4 h at 37°C. The loading mixture was then aspirated and the cells were washed three times with buffer (15 mM HEPES, 140 mM NaCl, 2 mM KCl, 1 mM MgSO₄, 1.8 mM CaCl₂, 11 mM glucose, pH 7.4; 1 ml/well) for 30 s, 5 min, and 30 s, respectively. One milliliter of buffer, with or without compounds to be tested, was then added to each well. After incubation for 2 min, the assay buffer was collected for measurements of ⁸⁶Rb⁺ released from the cells. Cells were then lysed by adding 1 ml of 100 mM NaOH to each well, and the lysate was collected for determination of the amount of ⁸⁶Rb⁺ that was in the cells at the end of the efflux assay. Radioactivity of assay samples and lysates was measured by liquid scintillation counting. Total loading (cpm) was calculated as the sum of the assay sample and the lysate of each well. The amount of ⁸⁶Rb⁺ efflux was expressed as a percentage of ⁸⁶Rb⁺ loaded. Stimulated ⁸⁶Rb⁺ efflux was defined as the difference between efflux in the presence and absence of nicotine.

Experiments with antagonists were done in two different ways. For obtaining an IC₅₀ value, inhibition curves were constructed in which different concentrations of an antagonist were included in the assay to inhibit efflux stimulated by 100 μ M nicotine. For determination of the mechanism of antagonist blockade, concentration-response curves for receptor activation by nicotine were constructed in the presence or absence of an antagonist. The maximal nicotine-stimulated ⁸⁶Rb⁺ efflux (E_{max}) was defined as the difference between maximal efflux in the presence of nicotine and basal efflux. EC₅₀, E_{max} , and IC₅₀ values were determined by nonlinear least-squares regression analyses (GraphPad, San Diego, CA).

Ligand Binding Studies. The ability of compounds to compete for the agonist recognition site of nAChRs was determined in ligand

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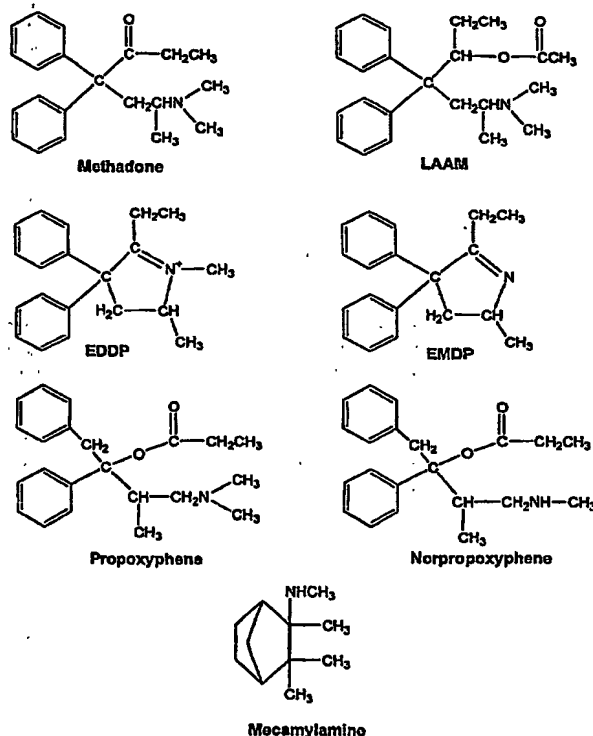


Fig. 1. The chemical structures of methadone, its metabolites, analogs, and mecamylamine.

binding studies, as described previously (Houghtling et al., 1995; Xiao et al., 1998). Briefly, membrane preparations were incubated with [³H]EB for 4 h at 24°C. Bound and free ligands were separated by vacuum filtration through Whatman GF/C filters treated with 0.5% polyethylenimine. The radioactivity retained on the filters was measured by liquid scintillation counting. Total binding and nonspecific binding were determined in the absence and presence of (-)-nicotine (300 μM), respectively. Specific binding was defined as the difference between total binding and nonspecific binding. Binding curves were generated by incubating a series of concentrations of each compound with a single concentration of [³H]EB. The IC₅₀ and K_i values of binding inhibition curves were determined by nonlinear least-squares regression analyses (GraphPad).

Results

Effects of Methadone on ⁸⁶Rb⁺ Efflux from KXα3β4R2 Cells. As shown in Fig. 2, at concentrations up to 1 mM, methadone did not increase ⁸⁶Rb⁺ efflux from KXα3β4R2 cells. In parallel assays, however, 100 μM nicotine stimulated ⁸⁶Rb⁺ efflux approximately 10-fold over basal levels, and this stimulation was completely blocked by 200 μM methadone.

Potency of Methadone and Its Enantiomers in Inhibiting Nicotine-Stimulated ⁸⁶Rb⁺ Efflux from KXα3β4R2 Cells. The potencies of racemic methadone and its enantiomers as antagonists of the nAChRs were examined by measuring ⁸⁶Rb⁺ efflux stimulated by 100 μM nicotine in the presence of increasing concentrations of the compounds. As illustrated in Fig. 3, racemic methadone potently inhibited nicotine-stimulated ⁸⁶Rb⁺ efflux in a concentration-dependent manner with an IC₅₀ of approximately 2 μM. Moreover, (+)-methadone and (-)-methadone inhibited the function of these receptors with similar potencies (Fig. 3; Table 1).

Low Affinities of Methadone for nAChR Agonist Binding Sites. We next examined the ability of methadone to compete for α3β4 receptor agonist recognition sites labeled by [³H]EB in membranes from KXα3β4R2 cells. As shown in Fig. 4, methadone does not compete effectively for [³H]EB binding sites. Thus, even at the highest concentration used (1 mM), methadone inhibited less than 50% of [³H]EB binding

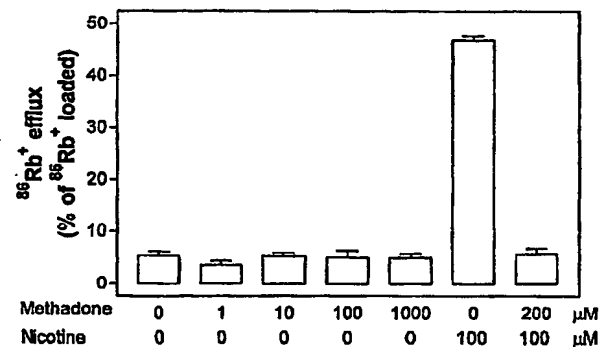


Fig. 2. Effects of methadone and nicotine on ⁸⁶Rb⁺ efflux from KXα3β4R2 cells. ⁸⁶Rb⁺ efflux was measured as described under *Experimental Procedures*. Cells were loaded with ⁸⁶Rb⁺ and then exposed for 2 min to buffer alone (to measure basal release), or buffer containing methadone at the concentrations shown, 100 μM nicotine or 100 μM nicotine plus 200 μM methadone. The ⁸⁶Rb⁺ efflux response was expressed as a percentage of ⁸⁶Rb⁺ loaded. Data shown are the mean ± standard error of four independent determinations.

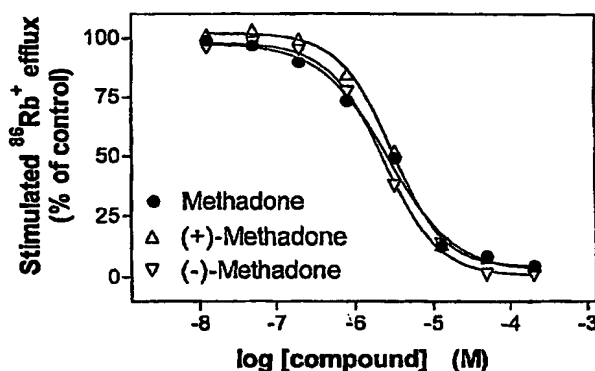


Fig. 3. Inhibition of nicotine-stimulated ⁸⁶Rb⁺ efflux from KXα3β4R2 cells by methadone and its two enantiomers. ⁸⁶Rb⁺ efflux was measured as described under *Experimental Procedures*. Cells were loaded with ⁸⁶Rb⁺ and then exposed for 2 min to buffer alone (basal release) or buffer containing 100 μM nicotine in the absence or presence of racemic methadone or one of the methadone enantiomers at the concentrations shown. ⁸⁶Rb⁺ efflux was expressed as a percentage of ⁸⁶Rb⁺ loaded, and control values were defined as ⁸⁶Rb⁺ efflux stimulated by 100 μM nicotine in the absence of methadone. Inhibition curves shown are from a single experiment measured in quadruplicate. See Table 1 for mean and standard error of the IC₅₀ values.

TABLE 1

Inhibitory properties of enantiomers of methadone and its metabolites and structural analogs of methadone on nicotine-stimulated ⁸⁶Rb⁺ efflux from KXα3β4R2 cells

IC₅₀ values were calculated from inhibition curves in which ⁸⁶Rb⁺ efflux was stimulated by 100 μM nicotine, as described under *Experimental Procedures*. Mecamylamine, a standard nAChR antagonist, was included for comparison. Data shown are the mean ± standard error of three to six independent measurements.

Drug	IC ₅₀ μM
(±)-Methadone	1.9 ± 0.2
(+)-Methadone	2.5 ± 0.2
(-)-Methadone	2.0 ± 0.3
(+)-EDDP	0.4 ± 0.2
(-)-EDDP	0.4 ± 0.1*
(+)-EMDP	5.8 ± 1.0
(-)-EMDP	6.3 ± 0.7
Propoxyphene	2.7 ± 0.4
Norpropoxyphene	1.8 ± 0.1
LAAM	2.5 ± 0.4
Mecamylamine	1.1 ± 0.2

* The IC₅₀ value for (-)-EDDP is significantly lower than that for mecamylamine ($p < 0.02$).

to α3β4 receptors. This was comparable with the weak binding potency of mecamylamine. In parallel assays carried out as positive controls, nicotine competed effectively for the agonist binding sites of α3β4 receptors, yielding a dissociation constant (K_d) of 560 nM, which is similar to that previously reported in these cells (Xiao et al., 1998). Methadone's very low affinity for the agonist recognition sites of α3β4 receptors contrasts with its high potency in blocking receptor function (IC₅₀ of about 2 μM) and suggests a noncompetitive mechanism of receptor antagonism.

Noncompetitive Block of nAChR Function by Methadone. To definitively identify the type of receptor blockade by methadone, we examined its effect on concentration-response curves for receptor activation by nicotine. As shown in Fig. 5, in the presence of 1 μM methadone, the maximum ⁸⁶Rb⁺ efflux stimulated by nicotine (E_{max}) was markedly

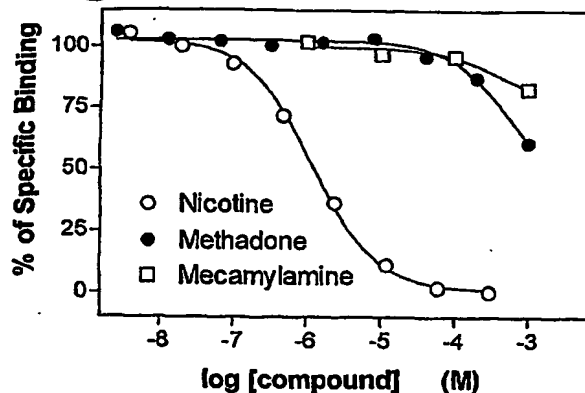


Fig. 4. Competition by methadone for [^3H]EB binding sites in membrane homogenates from KX α 3 β 4R2 cells. Binding assays were carried out as described under *Experimental Procedures* using 323 pM [^3H]EB. The K_i value for nicotine was 559 nM. The K_i values for methadone and mecamylamine cannot be estimated because there was less than 50% inhibition even at the highest concentration used (1 mM). Mecamylamine is shown for comparison.

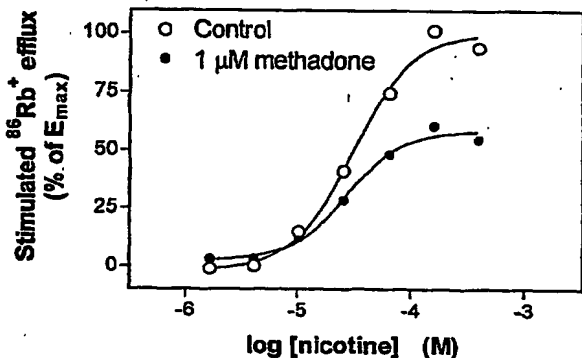


Fig. 5. Noncompetitive inhibition of nicotine-stimulated $^{86}\text{Rb}^+$ efflux from KX α 3 β 4R2 cells by methadone. $^{86}\text{Rb}^+$ efflux was measured as described under *Experimental Procedures*. Cells were loaded with $^{86}\text{Rb}^+$ and then exposed to buffer containing increasing concentrations of nicotine for 2 min in the absence (control) or presence of 1 μM methadone. The $^{86}\text{Rb}^+$ efflux was calculated as a percentage of $^{86}\text{Rb}^+$ loaded, and the E_{max} was defined as the maximum response in the absence of methadone. The curves shown are from a single experiment measured in quadruplicate. The EC_{50} values in the absence and presence of methadone were 28.8 ± 1.2 and 21.3 ± 2.1 μM , respectively (mean \pm standard error from four independent experiments). The E_{max} value (mean \pm standard error) in the presence of 1 μM methadone was $63 \pm 3\%$ of control values. Both the EC_{50} ($p < 0.05$) and E_{max} values ($p < 0.01$) in the presence of methadone are significantly different from control values.

reduced, but the EC_{50} for nicotine was altered only slightly, if at all. This result indicates that methadone does, in fact, block α 3 β 4 nAChR function primarily by a noncompetitive mechanism.

Inhibitory Effects of Methadone Metabolites and Structural Analogs on $^{86}\text{Rb}^+$ Efflux from KX α 3 β 4R2 Cells. We next tested seven compounds related to methadone, including its metabolites and structural analogs, for their agonist and antagonist effects on $^{86}\text{Rb}^+$ efflux from KX α 3 β 4R2 cells. At concentrations up to 100 μM , none of these compounds increased $^{86}\text{Rb}^+$ efflux (data not shown).

However, all of the compounds tested here were relatively potent blockers of nicotine-stimulated $^{86}\text{Rb}^+$ efflux (Table 1). Thus, the long-acting methadone analog LAAM as well as propoxyphene and norpropoxyphene were about as potent as methadone in blocking this α 3 β 4 receptor-mediated response. The methadone metabolite EDDP was even more potent; in fact, EDDP appears to be one of the most potent nAChR antagonists that has been reported, being about 5 times more potent than methadone and about twice as potent as mecamylamine (Fig. 6; Table 1). Furthermore, like methadone, the two enantiomers of the metabolites were equipotent in blocking α 3 β 4 nAChR (Table 1), although in these studies the difference in IC_{50} values between (-)-EDDP and mecamylamine was statistically significant ($p < 0.02$), while that for (+)-EDDP was not ($0.05 < p < 0.1$).

Noncompetitive Block of nAChR Function by Methadone Metabolites and Structural Analogs. None of the compounds examined here competed effectively for [^3H]EB binding sites (data not shown), suggesting that, like methadone, they block receptor function via a noncompetitive mechanism. To examine this more directly, we examined the effects of (+)-EDDP and LAAM on concentration-response curves for receptor activation by nicotine. As shown in Fig. 7, both of these compounds acted as noncompetitive blockers of α 3 β 4 nicotinic receptors.

Discussion

We investigated the effects of the enantiomers of methadone and its metabolites as well as three structural analogs of methadone on the function of rat α 3 β 4 nAChRs stably expressed in KX α 3 β 4R2 cells. All of these compounds inhibited nicotine-stimulated $^{86}\text{Rb}^+$ efflux in a concentration-dependent manner and with relatively high potencies, comparable with that of mecamylamine. In particular, EDDP, the major oxidative metabolite of methadone, with an IC_{50} of

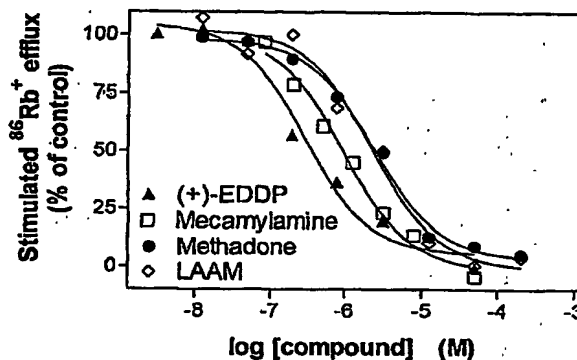


Fig. 6. Comparison of the inhibition of nicotine-stimulated $^{86}\text{Rb}^+$ efflux from KX α 3 β 4R2 cells by methadone, (+)-EDDP, LAAM, and mecamylamine. $^{86}\text{Rb}^+$ efflux was measured as described under *Experimental Procedures*. Cells were loaded with $^{86}\text{Rb}^+$ and then exposed for 2 min to buffer alone (basal release) or buffer containing 100 μM nicotine in the absence or presence of racemic methadone, (+)-EDDP, LAAM, or mecamylamine at the concentrations shown. $^{86}\text{Rb}^+$ efflux was expressed as a percentage of $^{86}\text{Rb}^+$ loaded, and control values were defined as $^{86}\text{Rb}^+$ efflux stimulated by 100 μM nicotine in the absence of methadone. Inhibition curves shown are from a single experiment measured in quadruplicate. See Table 1 for mean and standard error of the IC_{50} values.

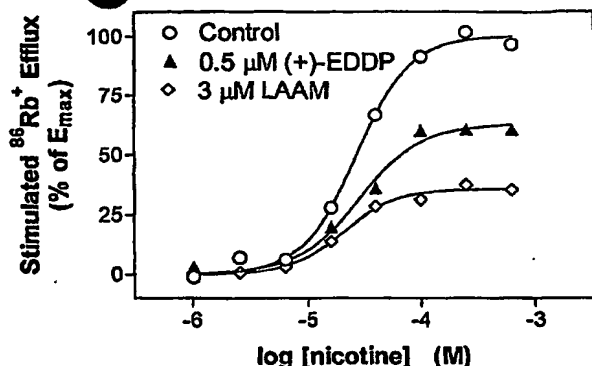


Fig. 7. Noncompetitive inhibition of nicotine-stimulated $^{86}\text{Rb}^+$ efflux from KXa3 β 4R2 cells by (+)-EDDP and LAAM. $^{86}\text{Rb}^+$ efflux was measured as described under *Experimental Procedures*. Cells were loaded with $^{86}\text{Rb}^+$ and then exposed to buffer containing increasing concentrations of nicotine for 2 min in the absence (control) or presence of 0.5 μM (+)-EDDP or 3 μM LAAM. The $^{86}\text{Rb}^+$ efflux was calculated as a percentage of $^{86}\text{Rb}^+$ loaded, and the E_{max} was defined as the maximum response in the absence of antagonists. The curves shown are from a single experiment measured in quadruplicate. The EC_{50} values for nicotine-stimulated $^{86}\text{Rb}^+$ efflux in the control cells, in the presence of 0.5 μM (+)-EDDP, and in the presence of 3 μM LAAM were, respectively, 28.2 ± 1.5 , 25.5 ± 1.5 , and $18.8 \pm 1.4 \mu\text{M}$. The E_{max} values in the presence of 0.5 μM (+)-EDDP and 3 μM LAAM were, respectively, $60 \pm 3\%$ and $44 \pm 5\%$ of control. Values are mean \pm standard error from three independent experiments. The values that were significant different from values of control are indicated by * $p < 0.05$ and ** $p < 0.01$, respectively.

about 0.4 μM , is one of the most potent nicotinic antagonists that has been reported.

A noncompetitive mechanism of nAChR blockade by methadone, EDDP, and LAAM is clearly indicated by the marked decrease in the maximum receptor-mediated response without a substantial change in the EC_{50} value for nicotine-stimulated $^{86}\text{Rb}^+$ efflux in the presence of these compounds. A noncompetitive mechanism is also consistent with the observation that neither methadone, its metabolites, nor its structural analogs competed effectively for [^3H]EB binding sites, which represent the agonist recognition site of the receptor. Taken together, these data indicate that all of these compounds most likely block within the $\alpha 3\beta 4$ nAChR channel. There also appeared to be a slight but statistically significant decrease in the EC_{50} value for nicotine-stimulated $^{86}\text{Rb}^+$ efflux in the presence of methadone and LAAM, implying that these drugs might actually increase the potency of nicotine at the receptor. Although it is very probable that the small difference in nicotine's EC_{50} values represents a statistical artifact, we cannot rule out an allosteric effect.

The (+)- and (-)-enantiomers of methadone and its metabolites are equipotent in blocking nAChR. This is in contrast to methadone's agonist actions at opiate receptors, which are ascribed almost entirely to its (-)-enantiomer. Therefore, the high potency of the (+)-enantiomers of methadone and its metabolites should allow blockade of nicotinic receptors without necessarily stimulating opiate receptors. This could then permit these (+)-enantiomers to be used in conditions where blockade of neuronal nicotinic receptors might be beneficial. For example, receptor blockade by mecamylamine is reported to aid in smoking cessation (Rose et al., 1994, 1998), and the most potent of the methadone metabolites is approximately twice as potent as mecamylamine. In addition, nicotinic re-

ceptors are thought to play a potentially important role in some analgesia pathways (Flores, 2000). Although analgesia has most often been associated with nicotinic agonists, these actions are incompletely understood and it is possible that nicotinic antagonists can also contribute to analgesia (Hamann and Martin, 1992). If this were the case for methadone and its metabolites, their analgesic effect through nicotinic mechanisms would perhaps be additive to analgesia mechanisms mediated by opiate receptors. This would be particularly useful where tolerance to opiates and/or ceiling effects are issues. In fact, both dextromethorphan, which blocks NMDA and nicotinic receptors, and (+)-methadone are reported to attenuate the development of tolerance to morphine analgesia (Elliott et al., 1994; Davis and Inturrisi, 1999).

The plasma concentration of methadone following a single dose is approximately 0.25 μM (Inturrisi and Verebely, 1972) and the steady-state concentration in patients taking methadone chronically can exceed 1 μM (de Vos et al., 1995; Alburges et al., 1996; Dyer et al., 1999). At these concentrations, methadone could be expected to produce significant blockade of $\alpha 3\beta 4$ nicotinic receptors. The steady-state plasma concentration of the more potent EDDP is usually much lower, but the peak concentration following administration of methadone can approach 0.2 μM (de Vos et al., 1995).

It should also be noted that (+)-methadone blocks NMDA receptor channels with potencies similar to, although slightly lower than, those found here at nicotinic receptors (Gorman et al., 1997; Stringer et al., 2000). Methadone's block of NMDA receptors also has been linked to its analgesic actions (Shimoyama et al., 1997; Davis and Inturrisi, 1999), and particularly to its potential usefulness for treating chronic and/or neuropathic pain (Elliott et al., 1995; Hewitt, 2000; Stringer et al., 2000). In addition, methadone's possible attenuation of morphine tolerance may involve NMDA receptors (Gorman et al., 1997; Davis and Inturrisi, 1999). In this regard, however, the block of nicotinic receptors by EDDP and (+)-methadone might also contribute directly to analgesic actions and even to the attenuation of morphine tolerance. Thus, it is possible that methadone and its metabolites can affect three different neurotransmission systems that have been associated with analgesia pathways and tolerance to opiates.

In conclusion, methadone, its metabolites EDDP and EMDP, as well as the methadone structural analogs LAAM, propoxyphene, and norpropoxyphene block $\alpha 3\beta 4$ nicotinic cholinergic receptors by a noncompetitive mechanism consistent with channel blockade. Both the (+)- and (-)-enantiomers of methadone and its metabolites are active; therefore, the high potency of the (+)-enantiomers of these compounds, particularly EDDP, in blocking nicotinic receptors should allow them to be used as probes of nicotinic receptors without affecting opiate receptors.

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